PREPARATION OF β-LACTOGLOBULIN C

Aschaffenburg and Drewry (1) determined that β-lactoglobulin of cow's milk occurs in at least two genetically determined variants designated as β-lactoglobulin A and β-lactoglobulin B. A third genetically determined variant, β-C, was recently discovered by Bell (3).

β-Lactoglobulins A, B, and C have been isolated and crystallized from the milk of homozygous cows by the method of Aschaffenburg and Drewry (2). The isolation of β-C has been modified by Kalan et al. (5, 6) because of its increased solubility.

More than 200 cows of the Jersey breed have been typed for the six possible β-lactoglobulin phenotypes: A, B, C, AB, AC, and BC. Of the 200, only two cows (one now deceased) have been found to produce homozygous β-C; this illustrates the low frequency of homozygous C. An examination of the milks of 105 Jersey cows of a single herd showed five heterozygote β-AC's and two heterozygote β-BC's.

A method for obtaining pure β-lactoglobulin C from a milk containing β-lactoglobulins A and C has been developed. The method employs a combination of Aschaffenburg's procedure and column chromatography. Piez et al. (7) have previously shown that mixtures of β-lactoglobulins A and B can be separated by DEAE-cellulose chromatography.

EXPERIMENTAL PROCEDURE

Fourteen and four-tenths liters of fresh, whole milk were obtained from a cow of β-lactoglobulin Type A/C. The milk was processed by the method of Aschaffenburg and Drewry (2), except that the fraction designated by them as P2 was processed twice, to increase the yields of β-lactoglobulin C.

From the fraction corresponding to F2 (2), supernatant and crystalline fractions were obtained amounting to 4.44 and 7.23 g and numbered 1 and 2, respectively, in the electrophoretic patterns shown in Figure 1, a. When the α-lactalbumin fraction, P2, was fractionated according to Aschaffenburg and Drewry (2), second P2 and F2 fractions were obtained. This F2 fraction yielded supernatant and crystalline fractions amounting to 2.62 and 0.86 g and numbered 3 and 4, respectively, in Figure 1, a. The second P2 fraction was processed to obtain the final supernatant and crystalline fractions amounting to 1.60 and 4.00 g and numbered 5 and 6, respectively, in Figure 1, a.

The weight of each fraction was determined spectrophotometrically at 280 mμ, using a specific extinction coefficient, $E_{1cm}^{1\%}$ of 9.4.

Agar-gel electrophoresis (4) was performed with approximately 0.5% protein solutions, adjusted to about pH 7.0. Each solution was applied to 1% agar (COLAB) in 0.025 M veronal buffer, pH 8.6. Each application was run on the same 3- by 4-in. glass plate for 2.5 hr at 300 v and 25 ma. After the run, the gel was stained for 2 to 3 min with Amido Black dye and was washed free of dye with several changes of 7% acetic acid.

Since the agar-gel electrophoresis showed only two fractions, no. 1 and 5, enriched with respect to β-lactoglobulin C, these fractions were combined, and the two proteins were separated by column chromatography, using DEAE-

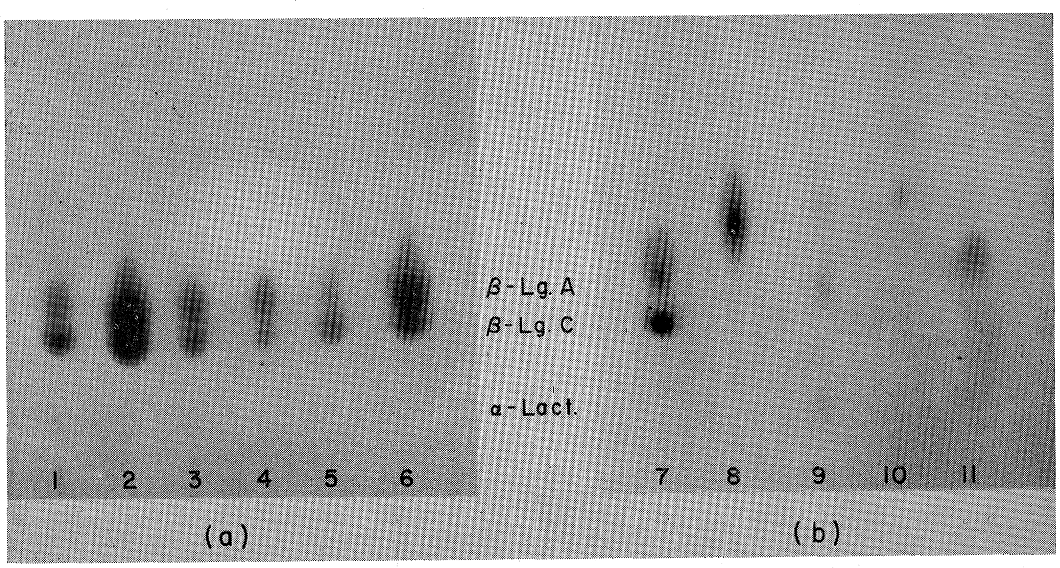

FIG. 1. Agar-gel electrophoresis of β-lactoglobulin fractions (see text for explanation). Approximately 0.5% protein solution, pH 7.0, in 1% agar (COLAB) in 0.025 M veronal buffer, pH 8.6. Run for 2.5 hr at 300 v and 25 ma. Gel stained with Amido Black and washed with 7% acetic acid.

cellulose (7) and an increaseing NaCl gradient in a constant phosphate buffer (5).

RESULTS AND DISCUSSION

The electrophoretic patterns in Figure 1, a show that fractions numbered 1 and 5 have more β-lactoglobulin C than β-lactoglobulin A, whereas Fraction 3 has about the same quantity of A as C. All other fractions are composed mostly of β-lactoglobulin A, since it is less soluble than C under these conditions.

Agar-gel electrophoresis (Figure 1, a) indicated that Fraction 3, the filtrate obtained after crystallization of β-lactoglobulin derived from the first fractionation of P2, gave approximately equal amounts of β-lactoglobulins A and C. After standing, this mother liquor yielded β-lactoglobulin A crystals. When crystallization was complete, the crystals were centrifuged, and the mother liquor was filtered. Ultraviolet absorption indicated 0.8 g β-lactoglobulin A crystals and 1.75 g β-lactoglobulins A and C in the mother liquor. Both the mother liquor and the crystals were applied to agar-gel for electrophoresis and were designated as no. 7 and 8, respectively (Figure 1, b). Fraction 7 illustrates the enrichment of β-lactoglobulin C, whereas Fraction 8 shows almost pure β-lactoglobulin A.

In Figure 1, b Fraction 7 (an AC mixture) does not correspond in mobilities to Fractions 8-11. This may be due to the presence of small amounts of salt in the sample, concentration of protein applied to the gel, or anomalies of protein migration when applied close to the edge of the gel.

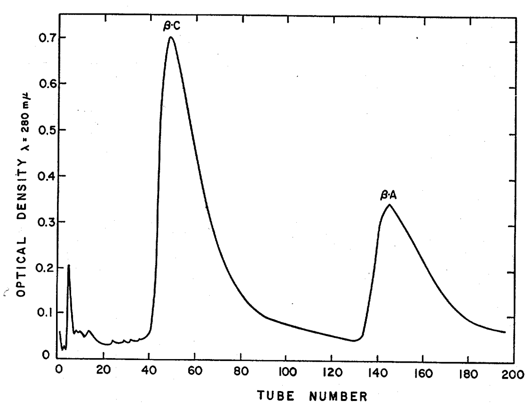

FIG. 2. Column chromatography of β-Lactoglobulins A and C. Five hundred forty milligrams previously dialyzed against phosphate buffer (0.05 M, pH 5.8) applied to a 1.8 by 35 cm DEAE-cellulose column. Elution accomplished at room temperature by an increasing NaCl gradient (seven chambers each of 500 ml of .01, .03, .05, .07, .09, .11, .14 M) in phosphate buffer (0.05 M, pH 5.8); 15 ml/tube.

The total yield of β-lactoglobulins A and C was 20.75 g, of which 11.67 g were obtained from Fractions 1 and 2 (Figure 1, a), whereas 44% of the total yield was derived from the α-lactalbumin fraction (P2).

Figure 2 illustrates two well-separated peaks after DEAE-cellulose chromatography of the combined fractions, designated no. 1 and 5. The fast-moving peak (Tubes 42-89) corresponds to β-lactoglobulin C as found by Kalan et al. (5). The slow-moving peak (Tubes 136-176) corresponds to β-lactoglobulin A. Eighty-two milligrams of β-lactoglobulin A and 185 mg of β-lactoglobulin C were finally recovered from a total of 540 mg applied to the column.

The pure β-lactoglobulin A (no. 10) and β-lactoglobulin C (no. 11) are illustrated in Figure 1, b, together with Fraction F1 (no. 9), which is the whey filtrate obtained after precipitation of the caseins with 20% Na_2SO_4.

In conclusion, it has been shown that it is possible to obtain β-lactoglobulin C in pure form from cow's milk containing β-lactoglobulins A and C. Since homozygous β-lactoglobulin C cows occurs only rarely, the present method makes it possible to obtain the protein in a relatively simple and direct manner.

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